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Instar Discrimination of Field-Collected Larvae Through Analysis of Frequency Distribution Curves of Head Capsule Widths Using the Program PeakFit

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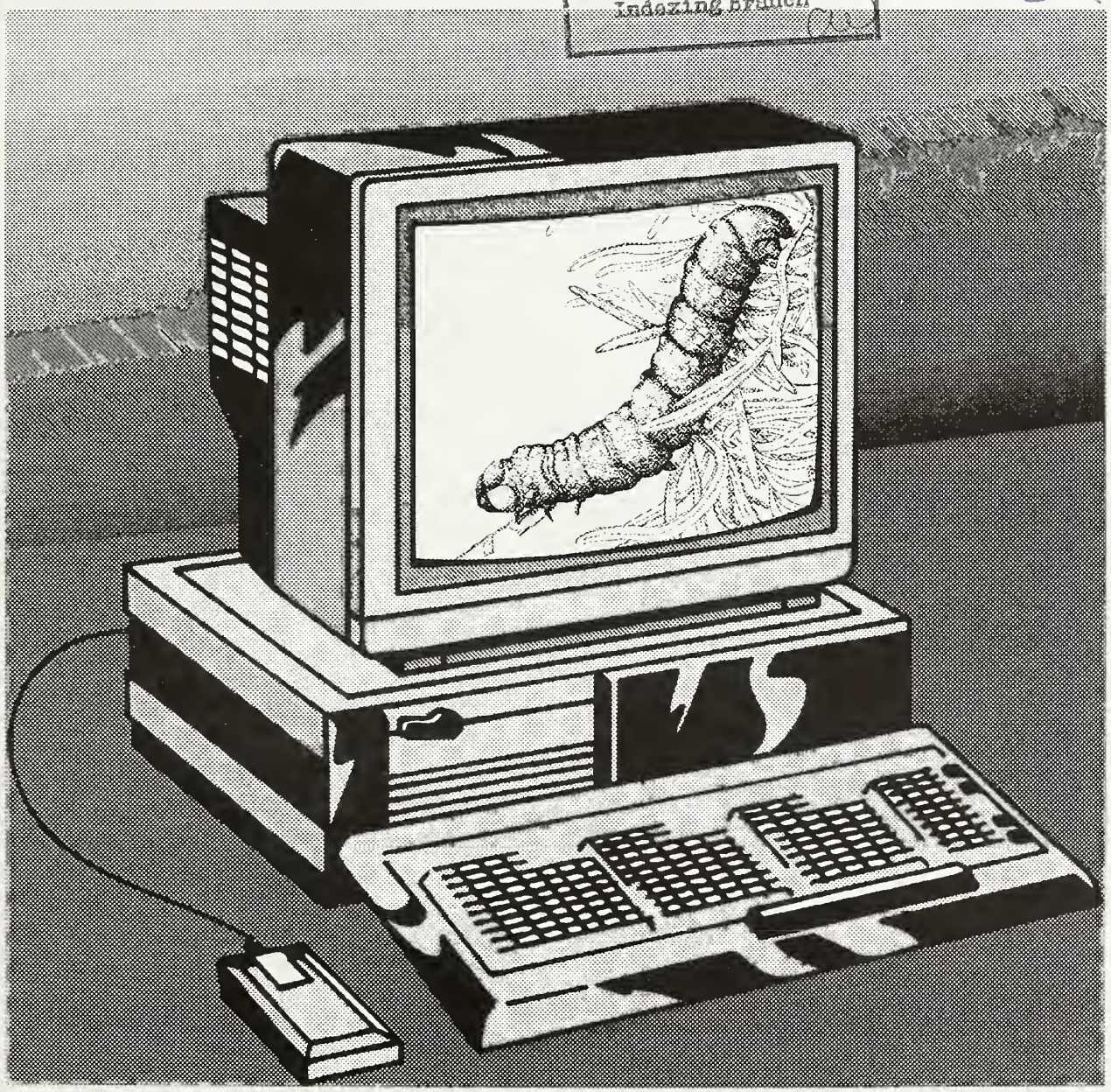

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Abstract

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A new method is described to analyze frequency distribution curves using a commercial program PeakFit™. It is illustrated with frequency distribution data of head capsule widths of the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), a species that exhibits developmental polymorphism. Analyses using PeakFit were capable of accounting for better than 99 percent of the number of head capsules in data sets of field-collected larvae and could classify from 50 to 73 percent to instar in the sets. Additional resolution is possible when the influence of parasitism on the head capsule width of the host is known. Such a possible influence was considered for *C. occidentalis* and, if the assumptions made were valid, up to 85 percent of samples could be characterized to instar. The advantages and disadvantages of using PeakFit are discussed.

Keywords: Insecta, instar discrimination, frequency distribution, head capsule widths, *Choristoneura occidentalis*, PeakFit.

Summary

This paper is a description of a new method to identify instars in a population using a sophisticated, commercial program called PeakFit™, a program written to address the needs of chemists, pharmacologists, engineers, statisticians, and others who employ nonlinear equations to solve curve-fitting problems. To my knowledge, this is the first time this program, or one like it, has been employed to solve an entomological or other biological problem. It allows one to analyze asymetric peaks of frequency distribution curves into their component parts, peaks such as those that might be generated by head capsule width measurements of insect larvae in a heterogeneously developing population.

Frequency distribution curves of head capsule widths of field-collected western spruce budworm (*Choristoneura occidentalis* Freeman) are used to illustrate the method. At a given time, larvae of this species can be found in the field in a number of apparently different instars and sizes. The species exhibits developmental polymorphism, wherein some larvae pass through six instars before pupating, others pass through seven instars, and still others pass through as many as eight or as few as five instars. All these develop at different rates and may be present in the population at the same time on a given host. The incidence of one of these instar groups over others in a population, and its economic significance, may be important, but unless all instar head capsules are accounted for over time for subsamples of individuals in a population, this estimate, and a conclusion of its significance, will remain moot. PeakFit ameliorates this dilemma by allowing one to make representation estimates of these instar groups at a given time and on larvae from a given host species. If the influence of parasitism on head capsule widths of a species like the budworm is known, it should also be possible to calculate the rate of parasitism in a population at a given time during larval development by using PeakFit.

Introduction

Entomologists have long recognized the value of knowing the distribution of instars within a population at a given time or to what instar a particular larva should be assigned. This knowledge is particularly important to economic entomologists, who are concerned with when to initiate control measures, and to students of life tables, who are concerned with what and when biological agents exert their highest efficacy against their insect hosts.

The most common method to estimate the total number of instars in a species, or to identify a particular instar, is by use of frequency distributions of head capsule widths, a method employed for over 100 years (Dyar 1890) and still widely used (for example, Got 1988, Jobin and others 1992, Rodriguez-Del-Bosque and others 1989, Russel and Bouzouane 1989, Shaffer and Rock 1983, Weatherby and Hart 1986, Whitfield and others 1987). In this method, the number of instars characteristic for a species corresponds to the number of peaks in the frequency distribution of head capsule widths measured from larvae collected throughout the postembryonic developmental period of the species.

This method should not be employed, however, without appropriate caveats. Parasitism, for example, frequently results in a reduction of the head capsule width of the host (for example, Hebert and Cloutier 1990, Iwantsch and Smilowitz 1975, McGugan 1955, Nealis 1987), and developmental polymorphism may result in some larvae within a population passing through more or fewer instars than the majority (Frick and Wilson 1981, Raske 1976, Schmidt and Lauer 1977, Schmidt and others 1977, Weatherby and Hart 1986). Among such larvae, the head capsule width in a given instar may be very different from that of other larvae in the same instar. Frequency distributions of head capsule widths in these instances may be complex and difficult to interpret, and may confound interpretations of instar compositions of a population.

The characterization of instars in the western spruce budworm (*Choristoneura occidentalis* Freeman [Lepidoptera: Tortricidae]) is particularly challenging because the univoltine species exhibits developmental polymorphism. In a given population, some larvae pass through six instars before pupating, others pass through seven instars, and still others pass through eight or as few as five instars (Schmidt and Lauer 1977, Schmidt and others 1977). Moreover, each instar group appears to develop at a different rate on different or even the same hosts under field conditions, similar to that found in *Acleris minuta* (Robinson), another tortricid species (Weatherby and Hart 1986). At a given time, it is therefore common to find larvae in a variety of instars, from the third through what appears to be the sixth instar. To further complicate an already complex situation, some of the larvae may be parasitized, which may retard development and influence larval size, as has also been found in other tortricid species (McGugan 1955, Nealis 1987). Because of these difficulties, instar representation and a mean instar estimate for a population of *C. occidentalis* at a given point in time cannot easily be ascertained by classical methods. A new method, described herein, allows one to not only break down resulting complex frequency distributions of head capsule widths from field-collected larvae of this species into their component peaks, or instars, but also to arrive at a mean instar

estimate for the population being monitored. This paper briefly presents some salient points of the commercial program called PeakFit[TM1] used in the method, presents evidence of the program's efficiency, and illustrates the method using head capsule widths of *C. occidentalis*.

Methods

Budworm Collections

Western spruce budworm larvae were collected in 1981-85 by teams working for the Pacific Northwest Research Station, USDA Forest Service. Collections analyzed for this paper were segregated by host—either Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Mirb.) Franco) or grand fir (*Abies grandis* (Dougl. ex D.Don) Lindl.)—and analyzed separately to minimize possible head capsule size differences due to nutritional causes.

The data analyzed and presented for the purposes of this paper are from the Bally Mountain (ST) plot located in the Starkey Experimental Forest about 40 kilometers west-southwest of La Grande in eastern Oregon. Larvae were collected on a single day, 15 June 1983, and preserved in KAAD (Peterson 1948). Composite data from nine additional plots also are included here.

Over 12,700 head capsules were measured to the nearest 0.01 millimeter by using a stereoscopic, binocular microscope. Among these were 1,679 head capsules from the ST plot, 810 collected from Douglas-fir and 869 collected from grand fir hosts. For convenience in presentation, the head capsule widths are shown as width in millimeters times 100.

Frequency distributions of head capsule widths were in the form X = width in mm × 100 and Y = frequency of occurrence of each width, and were subsequently analyzed by using the program PeakFit.

PeakFit

PeakFit is a DOS-based program that allows for the resolution of peaks in curves by using a nonlinear, least-squares method, optimized by the Marquardt-Levenberg algorithm, with starting points determined by interactive graphic methods. Fitting functions that are either internal to the program or that may be customized and entered by the user are employed to further describe these peaks (Jandel Scientific 1990). Because frequency distribution peaks of head capsule widths usually can be fitted with a normal, or Gaussian, distribution (Caltagirone and others 1983, McClellan and Logan 1994), this internal fitting function of PeakFit was used to characterize all peaks in our data sets.

Frequency distributions of the head capsule widths often exhibited varying degrees of randomness or "noise." This noise was minimized by subjecting the data file to one of three smoothing options: (1) a Polynomial Interpolation (Poly) option, (2) a Fast Fourier Transform Filter (FFT) option, or (3) a Lowess smoothing option (Jandel Scientific 1990). Each of these options could be invoked to perform various degrees of smoothing. The Lowess smoothing option proved unsatisfactory for our analyses

[1]Jandel Scientific, Corte Madera, CA 94925. The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

because peaks were observed to shift as the percentage of smoothing changed. The Poly and FFT options, at 30 and 22 percent smoothing (Jandel Scientific 1990), respectively, proved very satisfactory and yielded results comparable to each other. For this reason, and for economy, most of the curves presented will be those of FFT 22 percent, or FFT22.

PeakFit allows up to eight peaks to be resolved at one time. If the data, shown graphically, suggest that more than eight peaks are present, regions of the data set distribution can be sectioned successively and the component peaks resolved sequentially until all peaks are fully characterized. Sectioning was usually required in analyzing our data.

When all peaks in a data set or section of data have been approximated by the user, PeakFit can be invoked to determine the best, least-squares fit of the sum curve overlaying the component peaks. Statistics for each of the component peaks and for the sum curve may, upon command, be generated by the program, including an ANOVA that is performed on the amplitude, center, and width of each and all component peaks under the sum curve in arriving at statistics for the sum curve. All analyses presented were highly significant ($P < 0.01$).

An integrated area also is presented for each component peak of the distribution, which reflects the number of head capsules included in that peak. By summing the integrated areas of each of the component peaks in the data set, an estimate of the total number of head capsules in the sample can be ascertained. And accordingly, the percentage apportioned to each peak also can be determined.

Instars

To determine the amount of a data set that could be explained through the PeakFit analysis, peak centers (that is, means of head capsule widths) found in the analysis were compared with mean width values (± 1 percent) that have been published for known *C. occidentalis* instars (Bean and Batzer 1957, Lyon and others 1972, Schmidt and Lauer 1977).

The mean instar index (I) for the subpopulation on each host was determined by multiplying the numerical value of each instar by its representation (that is, percentage) in the subpopulation, summing these products, and dividing that product sum by the sum of the representations of all identified instars in the subpopulation:

$$I = \frac{\sum_{i=1}^{7} M_i P_i}{\sum_{i=1}^{7} P_i}$$

where

M_i = the instar represented in the population, and

P_i = the percentage of the population represented in each instar.

Data on the effects of parasitism on head capsule widths have not been published for *C. occidentalis*, but reductions probably do occur. McGugan (1955) and Nealis (1987) observed the phenomenon in other tortricid budworms due to parasitization by *Apanteles fumiferanae* Vier. and *Glypta fumiferanae* (Vier.)—McGugan in *C. fumiferana* (Clem.) and Nealis in *C. pinus pinus* Free (both parasite species also parasitize *C. occidentalis*). The percentages of reduction calculated from the difference of the means of parasitized instar widths from the means of corresponding instars of unparasitized widths for the two studies are as follows:

	***C. fumiferana* (McGugan 1955)**		***C. pinus pinus* (Nealis 1987)**
Instar	***Apanteles***	***Glypta***	***Apanteles***
		Percent	
III			-1.36
IV	-3.0	-4.4	-2.23
V	-21.5	-14.0	-7.61
VI		-20.3	-21.57

To determine if some of the PeakFit peaks may have been due to parasite-induced width reductions, assuming that similar reductions could and do occur in *C. occidentalis*, the published head capsule width values of Bean and Batzer (1957), Lyon and others (1972), and Schmidt and Lauer (1977) were reduced by the above listed percentages and the resulting values (± 1 percent) then compared with peaks found after PeakFit analysis.

In addition to the width reductions found by McGugan (1955) and Nealis (1987), Hebert and Cloutier (1990) observed reductions in larval widths of *C. fumiferana* following parasitization by *Meteorus* spp. But McGugan (1955:185) states that *Meteorus* "has a much less pronounced effect on the head-capsule size of the host." For that reason, width reductions presumed to be attributable to *Meteorus* were not investigated in this study.

Results and Discussion

PeakFit Curves and PeakFit Efficacy

Figures 1 and 2 show unsectioned head capsule width data sets of larvae collected on a single date from the ST plot before and after smoothing by PeakFit. The multi-modal character of the frequency distributions suggest the presence of developmental polymorphism or substantial parasitism and a host effect, or both. Despite the fact that some of the sculpture of the sum curves appears to be modified in the smoothing steps, resolution of the data sets after sectioning usually resolved the same major peaks in similar locations and comparable integrated areas for a given host.

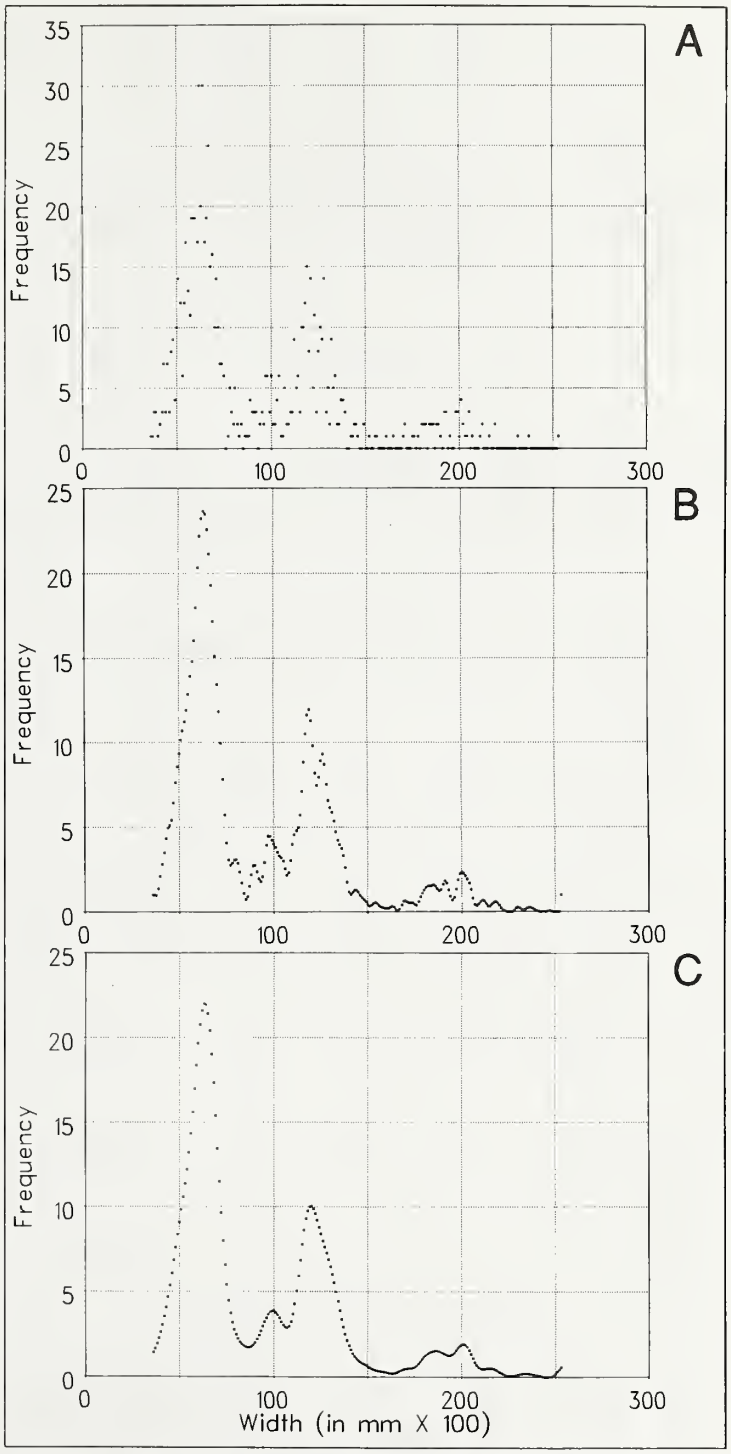


Figure 1—**A**: Unsmoothed and unsectioned ST plot data set of head capsule widths of larvae off Douglas-fir hosts. **B**: The same data set but smoothed with PeakFit's Poly30 option. **C**: The same data set but smoothed with PeakFit's FFT22 option.

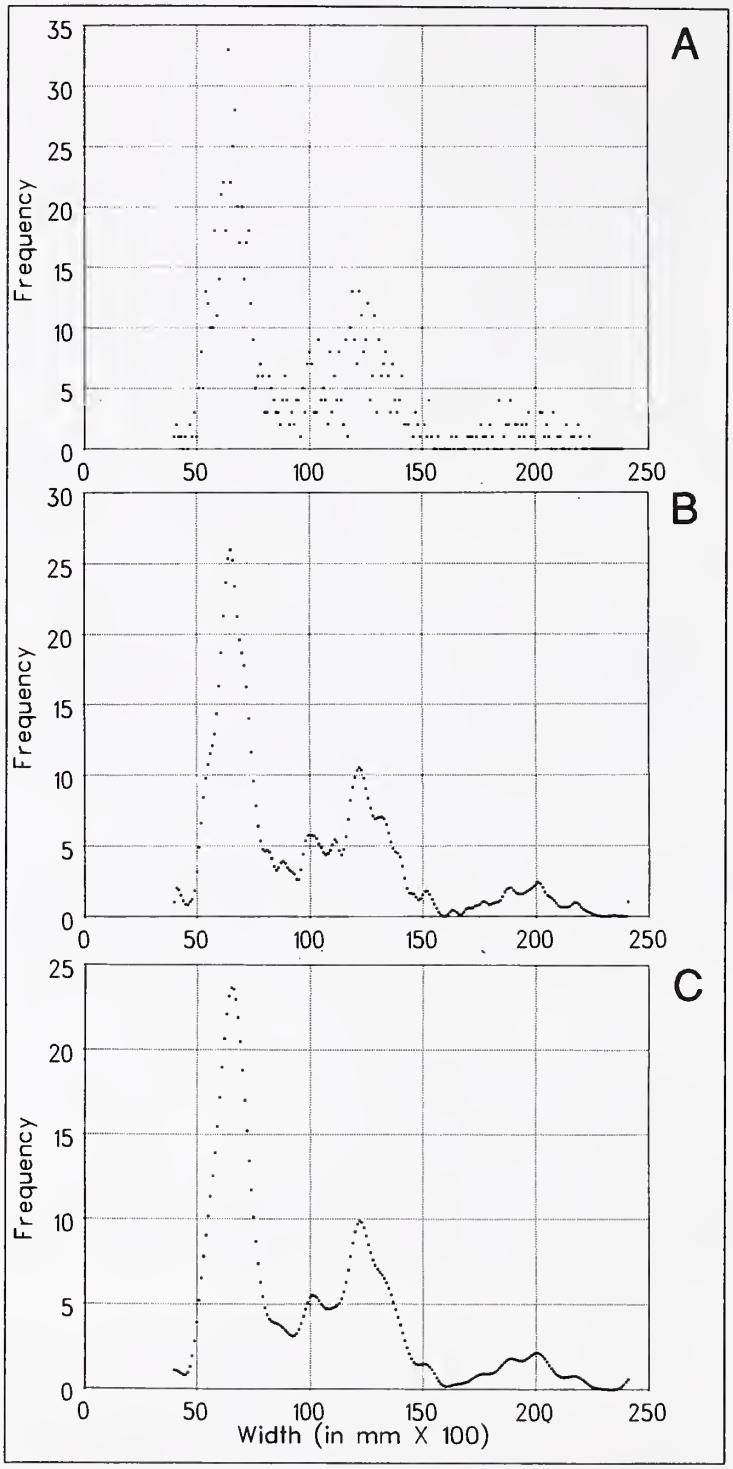


Figure 2—**A**: Unsmoothed and unsectioned ST plot data set of head capsule widths of larvae off grand fir hosts. **B**: The same data set, unsectioned but smoothed with PeakFit's Poly30 option. **C**: The same data set, unsectioned but smoothed with PeakFit's FFT22 option.

Table 1—ANOVA results for the sum curves shown in figures 3 and 4 (ST plot data)

Host[a]	Fig. no.	Figure position	Total peaks	Coefficient count	Fitted count	s.e. of curve	r^2	Source	Sum of squares	DF	Mean square	F
D-f	3	Top	6	18	18	0.09477	0.99988	Regres	2,333.816	17	137.28332	15,285.2
								Error	0.2874057	32	0.0089814	
								Total	2,334.104	49		
		Middle	6	18	18	.03759	.99988	Regres	696.72802	17	40.984001	29,006.6
								Error	.0861880	61	.0014129	
								Total	696.81421	78		
		Bottom	6	18	18	.00449	.99996	Regres	27.572274	17	1.6218985	80,367.5
								Error	.0012310	61	2.018 -05	
								Total	27.573505	78		
GF	4	Top	5	15	15	.15183	.99970	Regres	2930.2411	14	209.30294	9,079.8
								Error	.8759538	38	.0230514	
								Total	2931.1171	52		
		Middle	6	18	18	.04946	.99973	Regres	432.3179	17	25.43047	10,397.5
								Error	.1149533	47	.0024458	
								Total	432.43285	64		
		Bottom	6	18	18	.00181	.99999	Regres	28.125714	17	1.65445	505,061.0
								Error	.0001703	52	3.276 -06	
								Total	28.125885	69		

[a] D-f = Douglas-fir; GF = grand fir.

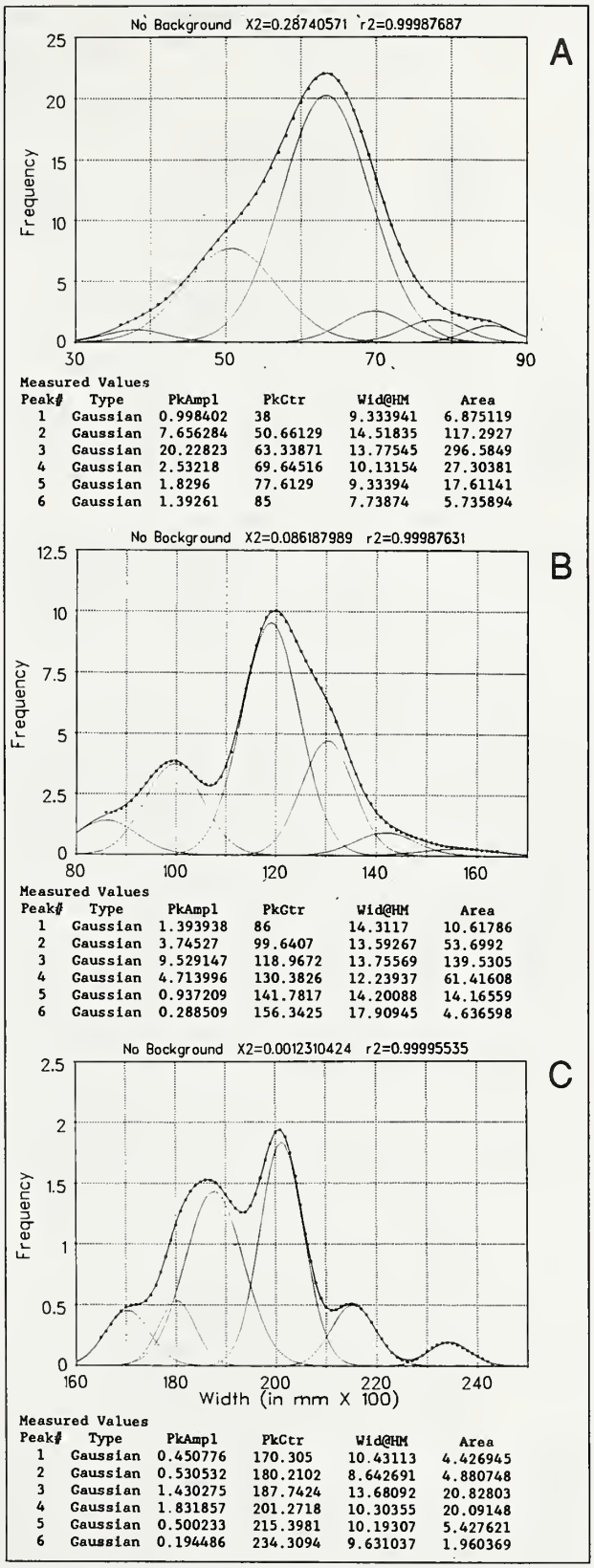
Figure 3 shows the results of the sectioned head capsule width data for larvae from Douglas-fir hosts on the ST plot after FFT22 smoothing by PeakFit; figure 4 shows similar sectioned data for larvae from grand fir hosts. In addition to the sum curves shown, the figures also show the component peaks. Although up to eight peaks could be resolved in a given section being analyzed, only five or six peaks were necessary to arrive at highly significant sum curve fits for these data set sections. Table 1 shows the ANOVA results for each of the sum curves in figures 3 and 4.

The number of head capsules accounted for after analysis with PeakFit was high, usually > 99 percent. Table 2 shows the percentages of head capsules accounted for following analyses of 4 unsectioned data sets and 10 sectioned data sets of each host. The ST data sets for the two hosts were included in the 10-plot sectioned analyses shown in table 2. Head capsules accounted for in the ST data sets also were considered high for both hosts. For example, of the 810 head capsules from larvae off Douglas-fir, 99.52 percent (806.11 capsules) were accounted for after Poly30 smoothing and subsequent sectioning, and 100.15 percent (811.22) were accounted for after FFT22 smoothing. Of the 869 head capsules in the ST set off grand fir, 100.19 percent (870.65) were accounted for after Poly30 smoothing, and 99.25 percent (862.48 capsules) were accounted for after FFT22 smoothing.

Although the accountability of head capsules in the data sets was considered high in all mean analyses, sectioned or unsectioned, unsmoothed or smoothed with either Poly30 or FFT22, smoothing with FFT22 was selected for subsequent analyses because it offered results with the least variation (table 2).

Instars

Peak centers (that is, mean instar widths) found in the PeakFit analyses of the ST plot data (figs. 3 and 4) were compared with published values (± 1 percent) of known instars for *C. occidentalis* (table 3). The published head capsule width values of Schmidt and Lauer (1977) accounted for 67.3 percent and 73.4 percent of the head capsules in the samples analyzed from Douglas-fir and grand fir hosts, respectively. The Bean and Batzer (1957) values accounted for up to only 2.5 percent of the ST samples, and those of Lyon and others (1972) accounted for 44.8 percent and 11.2 percent of the ST samples from the same hosts, respectively. Because the Schmidt and Lauer study was done in the laboratory, parasitism had no influence on their head capsule width measurements. It may be inferred that up to 67.3 percent of the budworms from Douglas-fir and 73.4 percent from grand fir hosts on the ST plot were probably not parasitized in 1983.



Measured Values

Peak#	Type	PkAmpl	PkCtr	Wid@HM	Area
1	Gaussian	0.998402	38	9.333941	6.875119
2	Gaussian	7.656284	50.66129	14.51835	117.2927
3	Gaussian	20.22823	63.33871	13.77545	296.5849
4	Gaussian	2.53218	69.64516	10.13154	27.30381
5	Gaussian	1.8296	77.6129	9.33394	17.61141
6	Gaussian	1.39261	85	7.73874	5.735894

Measured Values

Peak#	Type	PkAmpl	PkCtr	Wid@HM	Area
1	Gaussian	1.393938	86	14.3117	10.61786
2	Gaussian	3.74527	99.6407	13.59267	53.6992
3	Gaussian	9.529147	118.9672	13.75569	139.5305
4	Gaussian	4.713996	130.3826	12.23937	61.41608
5	Gaussian	0.937209	141.7817	14.20088	14.16559
6	Gaussian	0.288509	156.3425	17.90945	4.636598

Measured Values

Peak#	Type	PkAmpl	PkCtr	Wid@HM	Area
1	Gaussian	0.450776	170.305	10.43113	4.426945
2	Gaussian	0.530532	180.2102	8.642691	4.880748
3	Gaussian	1.430275	187.7424	13.68092	20.82803
4	Gaussian	1.831857	201.2718	10.30355	20.09148
5	Gaussian	0.500233	215.3981	10.19307	5.427621
6	Gaussian	0.194486	234.3094	9.631037	1.960369

Figure 3—**A**: Section of ST plot data set of head capsule widths from larvae off Douglas-fir hosts after smoothing with PeakFit's FFT22 option and showing the resolved component peaks (peak centers also noted in tables 3 and 4). **B**: The same as A but showing another section of the data set. **C**: The same as A but showing the last section of the data set.

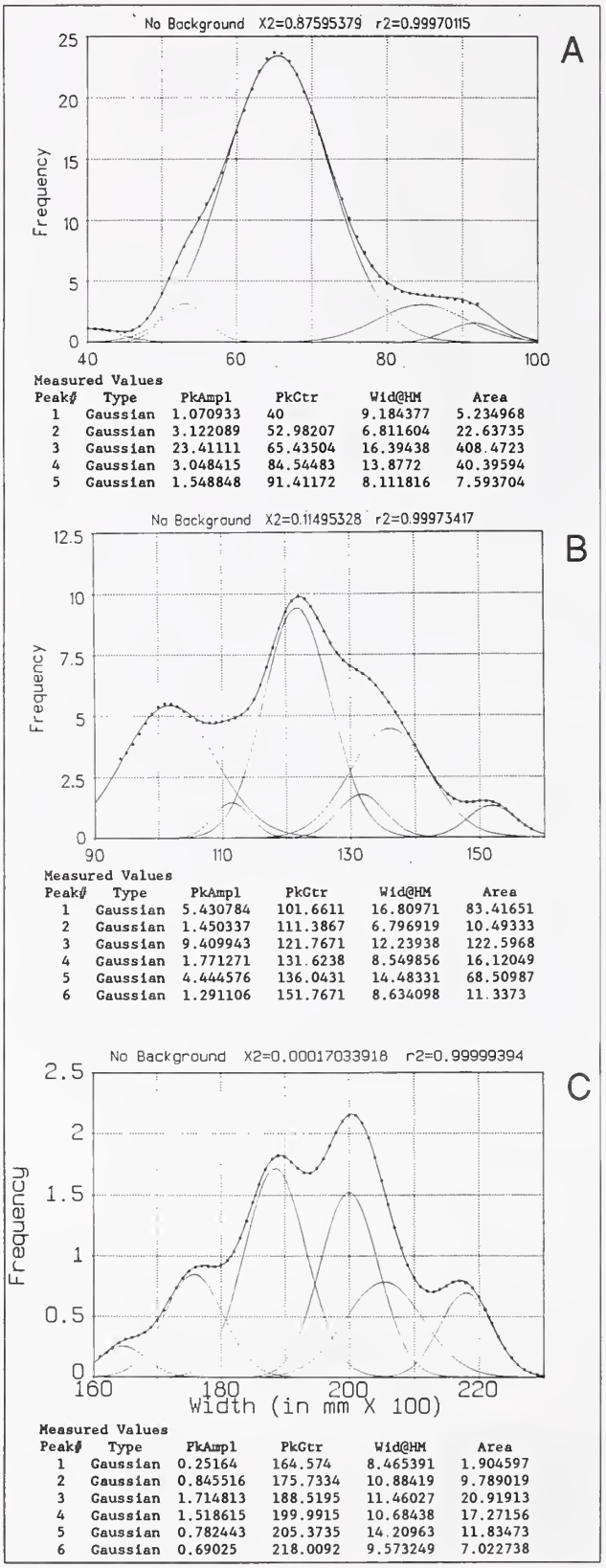


Figure 4—**A**: Section of ST plot data set of head capsule widths from larvae off grand fir hosts after smoothing with PeakFit's FFT22 option and showing the resolved component peaks (peak centers also noted in tables 3 and 4). **B**: The same as A but showing another section of the data set. **C**: The same as A but showing the last section of the data set.

Table 2—Percentage of head capsules accounted for in data sets following various PeakFit smoothing procedures and subsequent analyses

Host[a]	Unsectioned data sets: No. data sets	Mean no.caps in sets	Un-smoothed	Smoothing: Poly30	Smoothing: FFT22	Sectioned data sets: No. data sets	Mean no. caps in sets	Smoothing: Poly30	Smoothing: FFT2
			Percent					*Percent*	
D-f:	4					10			
$\bar{X}$		651.5	99.923	99.470	99.390		617.1	98.590	99.092
SD		66.656	0.4322	0.3680	0.2261		134.089	1.5886	1.3930
SE		33.328	.2161	.1840	.1131		42.403	.5024	0.4405
GF:	4					10			
$\bar{X}$		690.25	96.7675	99.7650	99.2675		659.40	99.2070	99.2640
SD		28.826	8.1595	1.2933	.2176		100.2666	1.2404	1.0309
SE		14.413	4.0798	.6467	.1088		31.7071	.3922	.3260

[a] D-f = Douglas-fir; GF = grand fir.

The mean instar index (I) for each of the ST plot host subpopulations is shown in table 3. The larval sample for *C. occidentalis* usually is conducted on and directed toward the fourth instar, and the 1983 sample on the ST plot was no exception. Thus, the estimates of I = 6.0, as determined from values presented in Bean and Batzer (1957), obviously are erroneous. The I-indices derived from instar values presented in either Lyon and others (1972) or Schmidt and Lauer (1977) comparisons are in the 4.07 to 4.38 range and, therefore, have more credence. *Choristoneura occidentalis* in the West, generally, and on the ST plot, specifically, develop more rapidly on grand fir than on Douglas-fir hosts. One, therefore, would expect I-values to be higher for larvae from grand fir than for those from Douglas-fir from the same site and in the same sample. This is reflected in the Schmidt and Lauer I-values of 4.29 and 4.07 from grand fir and Douglas-fir, respectively, but not so on the Lyon and others indices. The I-values of the latter are 4.29 and 4.38 for grand fir and Douglas-fir, respectively. This suggests that the instar measurements published by Schmidt and Lauer (1977) may be the more reliable values for the species than those published by Bean and Batzer (1957) or Lyon and others (1972).

Table 3—Comparison of published head capsule width values in *C. occidentalis* with peaks found after FFT22 smoothing and PeakFit analysis of sectioned data sets

Plot	Host[a]	Peak centers[b] (from PeakFit)	Peak areas (percent of total sample)	Unparasitized instars represented in peaks		
				Schmidt and Lauer 1977	Lyon and others 1972	Bean and Batzer 1957
ST.3D	D-f	38.0	0.9			
		50.66	14.4		III	
		63.34	36.5	III & IV		
		69.65	3.4	IV		
		77.61	2.2		IV	
		85.00	.7			
		86.00	1.3			
		99.64	6.6			
		118.97	17.2	V	V	
		130.38	7.6	VI	V	
		141.78	1.7			
		156.34	.6			
		170.30	.5			
		180.21	.6		VI	
		187.74	2.6	V & VI	VI	
		201.27	2.5			VI
		215.40	.7			
		234.31	.2	VII		
		Total =	100.01 percent			
			Sum totals[c] =	67.3 percent	44.8 percent	2.5 percent
				I[d] = 4.07	I = 4.38	I = 6.0
ST.3G	GF	40.0	0.6	III		
		52.48	2.6	III		
		65.44	47.2	IV		
		84.54	4.7	IV		
		91.41	.9			
		101.66	9.6			
		111.39	1.2			
		121.77	14.2	IV		
		131.62	1.9		V	
		136.04	7.9	VI		
		151.77	1.3			
		164.57	.2			
		175.73	1.1	VI		
		188.52	2.4	V & VI		
		199.99	2.0		VI	VI
		205.37	1.4			
		218.01	.8			
		Total =	100.0 percent			
			Sum totals[c] =	73.4 percent	11.2 percent	2.0 percent
				I[d] = 4.29	I = 4.29	I = 6.0

[a] D-f = Douglas-fir; GF = Grand fir.
[b] See figures 3 and 4.
[c] Sum totals = presumed total percentages of unparasitized instars accounted for in the ST data set according to the published head capsule width means (± 1 percent) of the authors cited.
[d] I = mean instar for the sample based on the identified instars and their representations on the plot.

Possible Parasitized Instars

If parasitism can cause a reduction in head capsule width in *C. occidentalis*, as it does in other *Choristoneura* species, the accounting for additional peaks in the ST data is possible. Thus, when the percentage reductions of McGugan (1955) and Nealis (1987) were applied to published widths (± 1 percent) for *C. occidentalis*, and those values then compared with peak centers resolved through PeakFit analysis, the characterization of additional peaks of possible parasitized larvae were identified (table 4). About 85.5 percent of the instars in the ST plot from grand fir may be interpreted when these representations are added to the percentage totals of unparasitized instars (table 3).

Table 5 shows the accountable peaks in the ST plot data and composite information for 10 plots similarly analyzed by PeakFit after FFT22 smoothing. It is apparent that the mean widths for *C. occidentalis* of Bean and Batzer (1957) are in very poor agreement with those obtained through PeakFit analysis. Those published by Schmidt and Lauer (1977) were in the best agreement, and those published by Lyon and others (1972) were intermediate but substantially less than those in Schmidt and Lauer. It is possible that an even greater percentage of accountable peaks might have been resolved if the actual effect of parasitism on head capsule widths were known in *C. occidentalis*.

It was of interest to see if the percentages of head capsule widths of parasitized larvae as ascertained through PeakFit analysis agreed with the actual percentages of parasitism found in larval rearings. A subsample of larvae was collected from the ST plot in 1983 and reared in the laboratory to assess the rate of parasitism. The subsample was made at the same time that the sample, subsequently analyzed by PeakFit, was collected. These results and comparisons are shown in table 6.

Many of the values of presumed parasitized larvae found through PeakFit analysis are low in comparison with the percentages of larvae found in the laboratory (table 6). PeakFit-derived values are from head capsule widths that could unequivocably be assigned to parasitized larvae if the degree of width reductions found by McGugan (1955) or Nealis (1987) could be applied to *C. occidentalis*. When these reductions were applied to published widths (re. Schmidt and Lauer 1977), and the results of the PeakFit analysis examined, some of the resulting widths of parasitized larvae coincided with the published widths of the unparasitized larvae. For example, included in the 67.3 percent of unparasitized larval widths accounted for in the Douglas-fir data set may be up to 57.1 percent of widths from parasitized individuals (see tables 3 and 5). These widths coincided with those of unparasitized individuals. Likewise, included in the 73.4 percent of accountable unparasitized widths of larvae off grand fir hosts may be up to 47.2 percent whose peak widths coincided with, and could have been attributable to, those of parasitized individuals. To avoid duplications, these values were included in the unparasitized category in table 5, despite the fact that a small portion of these peaks may have been attributable to head capsules from parasitized larvae. Nevertheless, the total data set percentages of all tree sources in table 5 for the parasitized plus unparasitized categories (that is, unpar + par [M or N]) should be valid.

Table 4—Comparison of possible additional parasitized larvae of *C. occidentalis* with peaks found after FFT22 smoothing and PeakFit analyis of sectioned data sets

Plot	Host[a]	Peak centers[b] (from PeakFit)	Peak areas (percent of total sample)	Additional possible parasitized instars represented in peaks[c]		
				Schmidt and Lauer 1977	Lyon and others 1972	Bean and Batzer 1957
ST.3D	D-f	38.0	0.9			
		50.66	14.4			
		63.34	36.5			
		69.65	3.4			
		77.61	2.2			
		85.00	.7	A (N)		
		86.00	1.3	A (N)		
		99.64	6.6	A & G (M)	A (M)	A (M)
		118.97	17.2			
		130.38	7.6			
		141.78	1.7	G (M)	A (N) & G (M)	
		156.34	.6	A (N) & G (M)	A (N)	
		170.30	.5			
		180.21	.6			
		187.74	2.6			
		201.27	2.5			
		215.40	.7			
		234.31	.2			
			Total = 100.01 percent			
		Sum totals:[d]	(N) Nealis	= 2.0 percent	2.3 percent	0.6 percent
			(M) McGugan	= 8.3 percent	8.9 percent	6.6 percent
ST.3G	GF	40.0	0.6			
		52.48	2.6			
		65.44	47.2			
		84.54	4.7			
		91.41	.9			
		101.66	9.6	A (N) & G (M)	G (M)	
		111.39	1.2	A (N) & G (M)		
		121.77	14.2		A (N)	
		131.62	1.9			
		136.04	7.9			
		151.77	1.3	A (N) & G (M)		
		164.57	.2			
		175.73	1.1			
		188.52	2.4			
		199.99	2.0			
		205.37	1.4			
		218.01	.8			
			Total = 100.0 percent			
		Sum totals:[d]	(N) Nealis	= 12.1 percent	14.2 percent	0 percent
			(M) McGugan	= 12.1 percent	9.6 percent	0 percent

[a] D-f = Douglas-fir; GF = grand fir.
[b] See figures 3 and 4.
[c] Parasite key: A = *Apanteles*, G = *Glypta*.
[d] Sum totals = possible additional percentages of parasitized instars accounted for in the ST data set according to the published (McGugan 1955, Nealis 1987) mean head capsule width reductions (± 1 percent) in other *Choristoneura* species.

Table 5—Percentage of data sets accounted for in the categories shown following PeakFit analysis[a]

Source of head capsule width values (± 1 percent)	Categories of data set(s) accounted for	ST plot		10-plot composite—mean values	
		D-f hosts	GF hosts	Douglas-fir hosts (± s.d.)	Grand Fir hosts (± s.d.)
		Percent			
Schmidt and Lauer (1977)	Unparasitized	67.3	73.4	50.586 ± 18.986	54.712 ± 24.324
	Additional parasitized (re. McGugan 1955 = M)[b]	8.3	12.1	16.173 ± 8.974	18.884 ± 23.251
	Unpar. + par.(M)	75.6	85.5	66.759 ± 14.870	73.596 ± 9.429
	Additional parasitized (re. Nealis 1987 = N)[b]	2.0	12.1	14.426 ± 12.822	17.937 ± 17.975
	Unpar. + par.(N)	69.3	85.5	65.012 ± 13.100	72.649 ± 12.083
Lyon et al. (1972)	Unparasitized	44.8	11.2	21.005 ± 10.625	22.680 ± 12.636
	Additional parasitized (M)[b]	8.9	9.6	13.970 ± 8.396	12.465 ± 10.984
	Unpar. + par.(M)	53.7	20.8	34.975 ± 13.353	35.145 ± 16.937
	Additional parasitized (N)[b]	2.3	14.2	12.523 ± 12.081	11.132 ± 10.078
	Unpar. + par.(N)	47.1	25.4	33.528 ± 14.783	33.812 ± 13.984
Bean and Batzer (1957)	Unparasitized	2.5	2.0	3.890 ± 4.680	4.160 ± 5.597
	Additional parasitized (M)[b]	6.6	0	3.850 ± 6.246	2.981 ± 3.269
	Unpar. + par.(M)	9.1	2.0	7.740 ± 8.303	7.141 ± 6.446
	Additional parasitized (N)[b]	0.6	0	6.300 ± 7.343	8.330 ± 9.869
	Unpar. + par.(N)	3.1	2.0	10.190 ± 8.449	12.490 ± 11.218

[a] All PeakFit analyses done after FFT22 smoothing.
[b] Additional peaks accounted for in PeakFit analyses according to possible head capsule width reductions in *C. occidentalis* when reduction factors for possible parasitized budworms were applied from McGugan (M; 1955) and Nealis (N; 1987) to instar source values.

Table 6—Additional possible percentage of parasitized instars represented in the PeakFit ST plot data from reductions in head capsule width data published by Schmidt and Lauer (1977) and compared with percentage (± s.e.) parasitized in the 1983 subsample of the population reared in the laboratory

Sample analysis	Source of reduction factors[a]	Parasites found or possible additional parasitized instars found in PeakFit[b]					
		Apanteles		*Glypta*		*Apanteles + Glypta*	
		Douglas-fir	Grand fir	Douglas-fir	Grand fir	Douglas-fir	Grand fir
		Percent					
Laboratory[c]	—	7.69 ± 3.31	6.43 ± 2.07	6.15 ± 2.98	12.86 ± 2.83	13.85 ± 4.28	19.30 ± 3.36
PeakFit	M	3.3	0	5.0	12.1	8.3	12.1
PeakFit	N	2.0	12.1	NA	NA	2.0	12.1

[a] M = McGugan 1955; N = Nealis 1987.
[b] N (Douglas-fir) = 65, N (grand fir) = 140.
[c] Subsample from ST plot collected in 1983, when the sample subsequently analyzed by PeakFit was obtained.

Conclusions

This study showed that the program PeakFit can be a useful method to characterize instars in a population by examining frequency distributions of larval head capsule widths. It can be particularly useful when the distributions are complex with considerable overlap of evident peaks. Thus, it can be a valuable tool in studying the phenomenon of developmental polymorphism in field populations. But if the overlap of peaks is minor, it might be just as efficient to apportion the overlap to the peaks in question by using previously published methods (Caltagirone and others 1983, Got 1988, McClellan and Logan 1986).

To characterize instars with efficacy, however, one should have a detailed knowledge of the number of instars and their respective mean widths that one would expect to find for the species. With this knowledge, it is possible to determine the proportion of representation for each instar in a population, as well as what mean instar the population is in at the time of sampling.

An approximation of the degree of parasitism in the population might also be ascertained under certain conditions without having to dissect or rear great numbers of larvae. The most efficacious parasite species must be identified, and the parasite species should have known and significant effects on head capsule widths of known instars of the host.

There are some disadvantages in using PeakFit to characterize larvae of a population. Much detailed information about the immatures of the species should be known before PeakFit analysis can be useful; information such as the number of instars characteristic for the species, expected means and variation of head capsule widths for each instar and sex, whether developmental polymorphism occurs, the effect of parasitism on head capsule widths, and so forth. Data sets should be fairly large. PeakFit measures component peaks of curves in frequency distribution presentations and, therefore, requires a substantial number of points. Most of the

data sets we examined exceeded 600 head capsule widths, but two data sets of 415 and 418 head capsules also gave us satisfactory results. The measurement of the "substantial number of points" and the construction of the data sets can be very time consuming, although the processing of these in PeakFit can be accomplished quickly once the program is mastered. And finally, because of the considerable amount of preparatory work necessary before PeakFit analysis, significant delays in obtaining and analyzing results may occur. Despite all these disadvantages, PeakFit can be a very useful qualitative and quantitative tool for investigating frequency distributions of head capsule widths and in characterizing insect populations for a given time. It also should be useful in resolving complex frequency distributions found in other applications, whatever they may be.

Epilogue

> When a series of measurements gives rise to a normal curve, we may probably assume something approaching a stable condition.... In the case of certain biological, sociological, and economic measurements there is, however, a well-marked deviation from this normal shape, and it becomes important to determine the direction and amount of such deviation. The asymmetry may arise from the fact that the units grouped together in the measured material are not really homogeneous. It may happen that we have a mixture of 2, 3,...*n* homogeneous groups, each of which deviates about its own mean symmetrically and in a manner represented with sufficient accuracy by the normal curve. Thus an abnormal frequency-curve may be really built up of normal curves having parallel but not necessarily coincident axes and different parameters. Pearson 1894:72

> In the case of a frequency-curve whose components are two normal curves, the complete solution depends in the method adopted in finding the roots of a numerical equation of the *ninth* order.... Clearly each component normal curve has three variables: (i) the position of its axis, (ii) its 'standard-deviation'..., and (iii) its area. Six relations between the given frequency-curve and its component curves would therefore suffice to determine the six unknowns. Innumerable relations of this kind can be written down, but, unfortunately, the majority of them lead to exponential equations, the solution of which seems more beyond the wit of man than that of a numerical equation even of the ninth order. Pearson 1894:75-76

> Ergo, the nexus between Pearson's sage comments, the "wit" of modern computer technology in the form of PeakFit, and the resolution of developmental polymorphism problems associated with complex frequency distributions of head capsule widths of some insect species.

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A new method is described to analyze frequency distribution curves using a commercial program PeakFit™. It is illustrated with frequency distribution data of head capsule widths of the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), a species that exhibits developmental polymorphism. Analyses using PeakFit were capable of accounting for better than 99 percent of the number of head capsules in data sets of field-collected larvae and could classify from 50 to 73 percent to instar in the sets. Additional resolution is possible when the influence of parasitism on the head capsule width of the host is known. Such a possible influence was considered for *C. occidentalis* and, if the assumptions made were valid, up to 85 percent of samples could be characterized to instar. The advantages and disadvantages of using PeakFit are discussed.

Keywords: Insecta, instar discrimination, frequency distribution, head capsule widths, *Choristoneura occidentalis*, PeakFit.

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